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Simplified Determinations of the "True" Creatinine Concentration in Serum and Urine

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Summary: The procedures using *Fuller*'s earth for the specific determination of creatinine were further simplified without loss of reliability. For routine purposes, deproteinization can be omitted if lipemic sera are excluded. The modification with deproteinization is recommended as a candidate for a selected method. Good correlation with an enzymatic procedure and the lack of relevant interference indicate a high analytical specificity.

The *Fuller*'s earth procedure is cheaper and more precise than the enzymatic method and slightly faster than the fading fraction method.

Urine samples should also be treated with *Fuller*'s earth before they are subjected to the *Jaffé* reaction.

Vereinfachte Verfahren zur Bestimmung der „wahren" Kreatinin-Konzentration

Zusammenfassung: Verfahren mit *Fuller*-Erde zur spezifischen Bestimmung der Kreatinin-Konzentration können ohne Verlust an Zuverlässigkeit weiter vereinfacht werden. Für Routinezwecke kann bei Ausschluß von lipämischen Seren auf die Enteiweißung der Proben verzichtet werden. Die Modifikation mit Enteiweißung wird als Kandidat für eine „ausgewählte Methode" empfohlen. Die gute Korrelation mit einem enzymatischen Verfahren und das Fehlen relevanter Interferenzen deuten auf eine hohe analytische Spezifität.

Das *Fuller*-Erde-Verfahren ist präziser als die enzymatische und etwas schneller als die fading fraction Methode.

Urinproben sollten vor der Durchführung der *Jaffé*-Reaktion mit *Fuller*-Erde behandelt werden.

Introduction

New modifications of known procedures are constantly being developed for the determination of creatinine in serum. In the recent years these methods have been based on either *Grafnetter*'s modification (1) of *Slot*'s fading fraction procedure (2), or one of the various adsorption techniques.

Several years ago *Knoll & Stamm* (3) published a careful study on a slight modification of *Owen*'s procedure (4) for the determination of the serum creatinine concentration. This method uses *Fuller*'s earth to adsorb almost all creatinine from an acid tungstate filtrate according to *Folin & Wu* (5). The results received are considered to be rather specific ("true creatinine").

The Dutch Society for Clinical Chemistry recommends an abbreviated procedure omitting the deproteinization (6). A similar procedure was suggested by *Knoll & Wisser* (7) also requiring five pipetting steps.

Recently *Lanser* et al. (8) reported that the Dutch method results in approximately 10% higher values than with the enzymatic method of *Wahlefeld* (9). No explanation was offered for this discrepancy.

In the present study, simplications of the method of *Knoll & Stamm* and of *Knoll & Wisser* are suggested which need less sample volume and fewer pipetting steps. In the former procedure, oxalic acid has been replaced by HCl. Both modifications were then compared with the original procedures, the fading fraction and an enzymatic method. It was intended to find out which modification applying *Fuller*'s earth is best suited to be recommended as a candidate for a selected method. Furthermore it was investigated whether urine samples should be treated with *Fuller*'s earth before performing the *Jaffé* reaction (10), since unexplained high creatinine clearance values were found in specimens from several patients.

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Materials and Methods

Instrumentation

Eppendorf Gerätebau GmbH (D-2000 Hamburg): microliter system 3000 consisting of microliter pipets, rotation mixer, microcentrifuge, digital photometer 5090, micro-flow-through-cuvette with tube pump 5260.

Materials

Lloyd's reagent (purified *Fuller*'s earth, Frankonit) was purchased from Roth AG (D-7500 Karlsruhe, No. 0109), Sigma Chemie GmbH (D-8000 Munich), Südchemie AG (D-8000 Munich) and Serva GmbH (D-6900 Heidelberg, catalogue No. 21940), all other materials from E. Merck (D-6100 Darmstadt).

Reagents for the modified method of *Knoll & Stamm* (3) as proposed below (method No. 2 under methods):

1. Tungstate (0.15 mol/l): 50 g $Na_2WO_4 \cdot 2H_2O$ (Merck No. 6673) and bidist. H_2O to 1 l.

2. Sulfuric acid (0.165 mol/l, Merck No. 9073).

3. *Fuller*'s earth suspension (6 g/l): 6 g *Fuller*'s earth, 10 ml HCl 1.0 mol/l (Merck No. 9057) and bidist. H_2O to 1 l.

500 µl of this suspension are filled into Eppendorf reaction cups (1.5 ml) either with a microliter pipet (500 µl) from a beaker or using a dispenser (e. g. Seripettor from Labora Mannheim, D-6800 Mannheim or Brand dispenser from K. Brand, D-6980 Wertheim). During this dispensing procedure, the suspension must be kept vigorously stirred (magnetic bar, high speed). If the reaction cups are carefully closed and kept in a refrigerator, the dispensed suspension can be stored for several months. Therefore, this step need not be performed during the actual assay procedure.

4. Picrate solution (12.4 mmol/l): mix 10 parts of 12 g/kg picric acid (Merck No. 604) and 35 parts NaOH 0.2 mol/l (Merck No. 9140). This solution is stable for at least 3 months if stored in a dark bottle.

5. Creatinine stock solution (8.34 mmol/l): dissolve 500 mg creatinine (Merck No. 5208, purity > 99%) in 5 ml HCl 1.0 mol/l (Merck No. 9057) and bidist. H_2O to 500 ml.

Although this solution is very stable, it is kept in a refrigerator and prepared every 2 months to avoid significant evaporation effects. A purer creatinine preparation can be obtained from the National Bureau of Standards (Washington, D. C., USA 20234, SRM No. 914, purity: 99.8%).

Note: Creatinine must be dried at 110 °C to constant weight.

6. Working standard solution (176.8 µmol/l = 2 mg/dl): 2 ml creatinine stock solution and bidist. H_2O to 100 ml. This solution is prepared at the beginning of each week.

Although the stability of the reagents is not critical (except the picrate solution) the adjective "indefinite" is avoided, since under routine conditions evaporation effects restrict the usage of most solutions to a definite period of time.

Reagents for the modified method of *Knoll & Wisser* (7) as proposed below (method No. 4 under methods):

1. *Fuller*'s earth suspension was prepared as described above.

2. Magnesium chloride solution (0.15 mol/l): 3.06 g $MgCl_2 \cdot 6H_2O$ (Merck No. 5833) and bidist. water to 100 ml (stable for several months).

3. Picrate solution (16.8 mol/l): 30 ml picric acid (12 g/kg, Merck No. 604), 10 ml NaOH (2 mol/l, Merck No. 9136), 20 ml $MgCl_2$ (0.15 mol/l) and bidist. water to 100 ml. This solution is stable for 3 months if stored in a brown bottle.

4. Creatinine standard solution (176.8 µmol/l) as described above.

Reagents for the determination of the creatinine concentration in urine:

1. The *Fuller*'s earth suspension and the picrate solution are the same as for method No. 4.

2. The standard solution (8.84 mmol/l) is identical to the creatinine stock solution decribed above.

Methods

The following procedures were applied in the present study:

Method No. 1 (with deproteinization and adsorption of creatinine to *Fuller*'s earth) was performed exactly as described by *Knoll & Stamm* (3).

Method No. 2 (with deproteinization and adsorption of creatinine to *Fuller*'s earth) is a simplification of method No. 1 as outlined below (DIN-Normentwurf 58973, Teil 1).

Method No. 3 (with adsorption of creatinine to *Fuller*' s earth without prior deproteinization) was performed exactly as described by *Knoll & Wisser* (7).

Method No. 4 (with adsorption of creatinine to *Fuller*'s earth without prior deproteinization) is a simplification of method No. 3 as outlined below.

Method No. 5: The fading fraction method was performed as described by *Grafnetter* et al. (1). Only one-tenth of the volumes recommended by the authors was used. Therefore, the Eppendorf microliter system could be applied (sample volume 200 µl).

Method No. 6: The SMA 12/60 procedure was performed as described by the Technicon Corp. using the *Jaffé* reaction after separation of proteins by dialysis.

Method No. 7: The enzymatic determination of the creatinine concentration in serum was performed according to *Wahlefeld* et al. (9) using the test combination No. 166414 from Boehringer Mannheim Corp. (D-6800 Mannheim).

Method No. 8: For the determination of the creatinine concentration in urine the method of *Taussky* was performed as described by the author (11) and compared with method No. 9.

Method No. 9 is a modification of method No. 4 applied to urine.

Pipetting scheme for method No. 2

Pipet into an Eppendorf reaction vessel:

Na-tungstate	100 µl
sample (serum, standard, bidist. H_2O)	100 µl
H_2SO_4	200 µl

mix 3 minutes, centrifuge 2.5 minutes (12000 *g*)

supernatant	300 µl
Fuller's earth suspension	500 µl

mix 1 minute; centrifuge 2.5 minutes (12000 *g*); aspirate supernatant with a needle connected to a suction pump. To the pellet add

alkaline picrate	500 µl

mix 15 minutes. Read absorbance of supernatant at Cd 509 nm with a flow-through microcuvet against reagent blank (H_2O instead of serum) 30 minutes after addition of picrate. Centrifuge 2.5 minutes before reading.

Calculation: $C = A \cdot F$

C = concentration of sample (µmol/l), A = absorbance of sample. The factor F is determined daily:

$$F = \frac{c_{st}}{A_{st}} = \frac{177}{A_{st}}$$

A_{st} = absorbance of standard solution, c_{st} = concentration of standard solution (176.8 µmol/l = 2.0 mg/dl). The standard solution is treated in the same way as serum samples.

A flow-through microcuvette (filling volume about 70 µl) is preferred, which allows a ratio between volumes for rinsing and measuring of 5:1. The percent interaction coefficient (12, 13) is about 0.1. In the concentration range up to 1700 µmol/l creatinine (20 mg/dl), which corresponds to the range of

linearity, carry-over effects can be neglected. If a suction microcuvette (filling volume approx. 200 µl) is used, the cuvette can only be prerinsed with 200 µl; then the interaction coefficient rises to about 5%.

The absorbance difference can also be measured at Hg 492 nm or between 480 and 510 nm. The highest absorbance difference was found at 482 nm.

Pipetting scheme for method No. 4

To Eppendorf reaction cups containing 500 µl *Fuller*'s earth suspension, add:

	Reagent blank	Standard	Specimen
Bidist. Water	100 µl	–	–
Standard solution	–	100 µl	–
Specimen	–	–	100 µl

Mix 5 minutes, centrifuge 1 minute (12000 *g*). Aspirate supernatant with a needle connected to a suction pump. To the pellet add

Alkaline picrate	500 µl	500 µl	500 µl

Mix 5 minutes, centrifuge 1 minute and read absorbance of supernatant at Cd 509 nm with a flow-through microcuvette against reagent blank. The calculation of the result is the same as for method No. 2.

For the determination of the creatinine concentration in urine (method No. 9), samples (including standard solutions) are diluted 1 + 50 (20 µl + 1000 µl bidist. H_2O) using an Eppendorf diluter. One hundred µl of this diluted sample are added to 500 µl *Fuller*'s earth suspension. Subsequent treatment was as described above for method No. 4. Finally, 1000 µl alkaline picrate solution are added to the pellet. After careful mixing and centrifugation (as for method No. 4), the absorbance of the supernatant was measured at Cd 509 nm (500–510 nm or Hg 492 nm) against a reagent blank. This procedure is linear up to 90 mmol/l creatinine.

Recovery experiment

The recovery of creatinine added to various serum samples was calculated from the results of the following assays:

1. 9.0 ml bidist. H_2O + 1.0 ml creatinine solution,
2. 9.0 ml serum + 1.0 ml creatinine solution, and
3. 9.0 ml serum + 1.0 ml bidist. H_2O.

The recovery is 100%, if the creatinine concentration of assay (2) equals assay (1) + (3).

Statistical procedures

The function of the linear regression, the coefficient of correlation and the standard deviation were calculated by conventional methods (15, 18). In addition, the regression line was compared with the standardized principle component (14, 15), which permits both variables (x_i and y_i) to be measured with random errors.

Results and Discussion

Imprecision

All methods with *Fuller*'s earth were performed with comparable between-days imprecision (tab. 1). The coefficient of variation varied from 1.8 to 5.9%. The procedure of *Knoll & Stamm* yielded the highest imprecision, which, however, was still lower than with the enzymatic method.

Inaccuracy

The creatinine concentration was determined in various unselected serum samples. The original method of *Knoll & Stamm* (3) correlated well with the simplified version proposed above as method No. 2 (fig. 1a, b). Therefore, the latter was preferred for further experiments.

Tab. 1. Precision of the creatinine determination in control sera.

	Within run imprecision				Between days imprecision			
	$\bar{x}$ [1] (µmol/l)	s (µmol/l)	CV (%)	n	$\bar{x}$ (µmol/l)	s (µmol/l)	CV (%)	n
A) *Fuller*'s earth procedure with deproteinization (method No. 1)								
Asid control serum (No. 405B)	147	2.52	1.7	(30)	146	7.05	4.8	(30)
Hyland reference serum (No. K00 2)	363	6.06	1.7	(30)	360	21.4	5.9	(30)
B) *Fuller*'s earth procedure with deproteinization (method No. 2)								
Asid control serum (No. 405B)	149	1.74	1.2	(19)	150	2.72	1.8	(19)
Hyland reference serum (No. K00 2)	388	3.16	0.8	(17)	388	10.2	2.6	(17)
C) *Fuller*'s earth procedure without deproteinization (method No. 4)								
Control serum Behringwerke (No. 116 L)	184	3.60	1.9	(15)	182	4.2	2.3	(15)
D) Fading fraction method (method No. 5)								
Asid control serum (No. 405 B)	149	4.8	3.2	(14)	148	6.5	4.4	(13)
Hyland reference serum (No. K00 2)	349	7.8	2.2	(14)	351	16.9	4.8	(13)
E) Enzymatic procedure (method No. 9)								
Asid control serum (No. 408 B)	168	21.3	12.6	(11)	168	25.7	14.6	(11)

[1]) mean value (µmol/l) with standard deviation and coefficient of variation (n = number of determinations).

Tab. 2. Comparison of various *Fuller*'s earth products for the determination of the creatinine concentration in control sera. All values are means of at least 2 experiments.

	Tonsil [1] Standard	Tonsil AC	Tonsil Optimum	Tonsil ACC	Sigma F-200	Sigma F-16	Sigma F-60	Serva	Roth
Standard solution (ΔA)[2])	0.217	0.211	0.242	0.226	0.091	0.103	0.066	0.205	0.201
Fluinorm (μmol/l)	118	115	118	115	132	127	123	125	120
Precinorm (μmol/l)	147	148	145	144	169	164	160	165	161
Monitrol I (μmol/l)	117	118	111	108	118	122	134	132	130
Hyland NO 2 (μmol/l)	78	76	77	74					

[1]) trade name of Südchemie
[2]) absorbance difference at Cd 509 nm

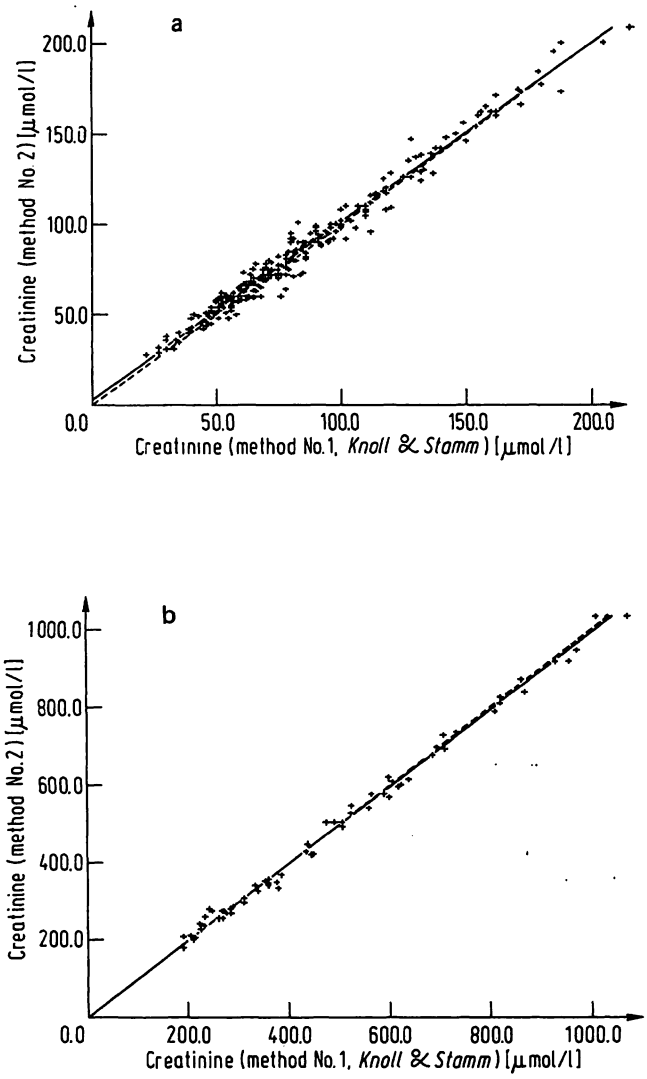

Fig. 1. The determination of the serum creatinine concentration with the method of *Knoll & Stamm* and the proposed modification. Standardized principle component:
a) $y = 0.98x + 3.35$, b) $y = 1.00x - 2.31$.
Linear regression analysis: a) $y = 0.99x + 2.73$, $r = 0.99$, $n = 240$; $\bar{x} = 83.24$ ($s = 35.86$), $\bar{y} = 84.76$ ($s = 35.07$); b) $y = 1.00x - 2.31$, $r = 1.00$, $n = 70$, $\bar{x} = 480.40$ ($s = 246.5$), $\bar{y} = 476.91$ ($s = 246.5$); the broken line represents the x/y relation, the drawn line the standardized principle component.

Fuller's earths from various commercial sources were compared for the creatinine determination using control sera (tab. 2). The preparations from Sigma yielded a relatively low absorbance difference indicating a poor recovery. The highest values were observed with Tonsil which was used in the comparison experiments. With samples from patients comparable results were obtained with various products from Südchemie (tab. 3), Serva and Roth (tab. 4). Increasing the HCl concentration in the

Tab. 3. Comparison of *Fuller*'s earth products of various quality from the same supplier (Südchemie AG, D-8000 Munic) for the determination of the creatinine concentration. All values are in μmol/l. (method No. 2).

Patient No.	Creatinine (μmol/l) Standard	AC	ACC	Optimum
1	96	96	104	90
2	162	159	168	164
3	402	408	398	400
4	40	32	39	34
5	70	75	80	71
6	172	176	170	170
7	908	910	862	901
8	847	853	838	825
9	60	65	78	66
10	74	68	74	70
11	270	271	263	257
12	400	406	408	407
13	52	46	42	45
14	101	105	107	110
15	111	118	114	118
16	268	275	268	274
17	932	950	930	915
18	68	72	70	71
19	80	86	84	79
20	116	112	111	117
21	640	658	660	652
22	573	572	572	578
23	478	480	468	452
24	82	80	81	82
25	75	79	80	79
26	64	65	62	62
27	74	78	78	78
28	68	70	73	71
mean value	260	263	260	259

Tab. 4. Comparison of *Fuller*'s earth products from various suppliers for the determination of the creatinine concentration. All values are in µmol/l. (method No. 2).

Patient No.	Creatinine (µmol/l) Tonsil [1]) Standard	Serva	Roth I 225020 S[2])	Roth II 2060206[2])
1	558	570	547	540
2	138	139	145	146
3	305	313	312	311
4	818	814	820	818
5	728	689	702	709
6	137	142	141	138
7	119	122	121	115
8	249	250	258	252
9	247	255	252	258
10	331	338	334	341
11	35	38	40	38
12	81	86	76	80
13	70	72	76	76
14	83	78	91	86
15	206	204	200	202
16	49	46	51	54
17	53	56	59	60
18	748	748	740	728
19	55	50	52	61
20	68	67	60	65
21	28	31	40	36
22	74	69	71	72
23	66	69	70	68
24	74	78	74	75
25	38	40	45	44
26	52	55	51	55
27	54	53	50	52
28	67	64	60	66
29	65	68	72	68
30	82	85	88	81
mean value	189	189	190	190

[1]) trade name of Südchemie
[2]) lot number

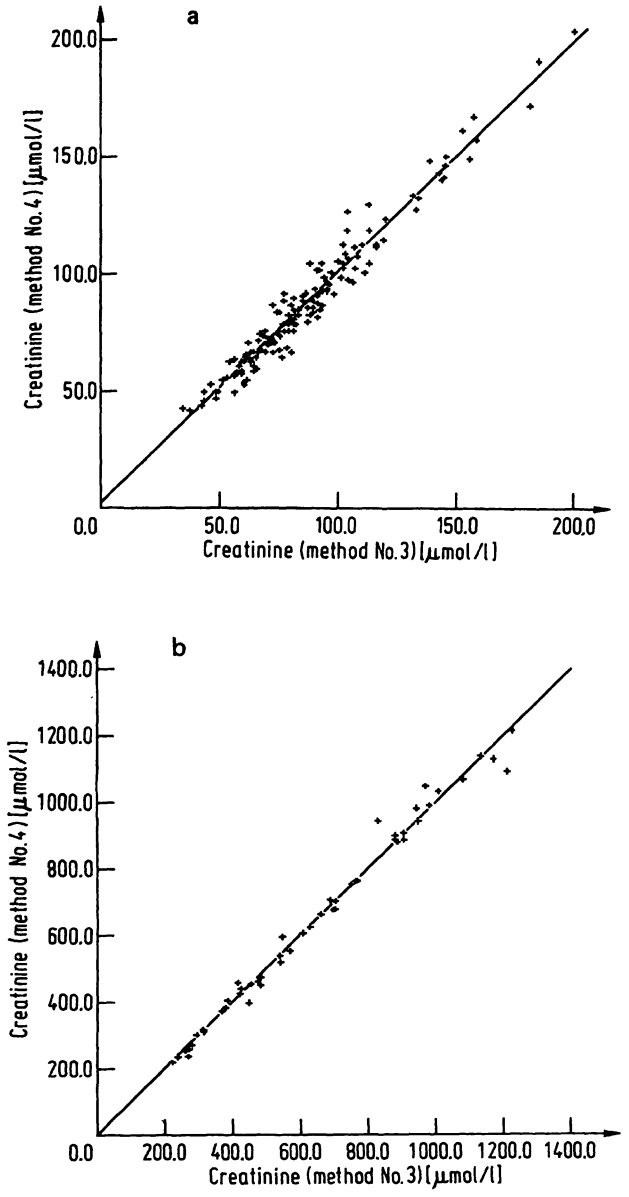

Fig. 2. Comparison of the creatinine concentration in various patient sera determined with the original method of *Knoll & Wisser* (method No. 3) and the modified procedure without deproteinization (method No. 4).
a) drawn line = linear regression analysis ($y = 0.98x + 1.97$; $r = 0.97$; $n = 166$); standardized principle component: $y = 1.01x + 0.30$; $\bar{x} = 87.3$ ($s = 29.2$); $\bar{y} = 87.6$ ($s = 29.4$); b) drawn line = linear regrsssion analysis ($y = 1.00x + 1.96$; $r = 0.99$; $n = 58$); standardized principle component: $y = 1.00x + 1.34$; $\bar{x} = 630.3$ ($s = 291.9$), $\bar{y} = 630.9$ ($s = 292.8$).

Fuller's earth suspension impaired the precision of the procedures proposed.

When the deproteinization step was omitted method No. 3 (method of *Knoll & Wisser*) also correlated well with the method No. 4 (fig. 2). However, in comparison with method No. 2 a mean error of about 7% was observed (fig. 3). This slight overestimation of the creatinine concentration could be accepted for clear sera, but it increased seriously in lipemic sera (tab. 5). Under these conditions, method No. 2 appeared not to be influenced by turbidity and correlated much better with method No. 6 which uses a dialysis step.

The difference of mean values between method No. 2 and No. 6 is not representative. In a separate experiment (not shown) about 12% higher results were obtained with the SMA procedure (mean value = 116 µmol/l, n = 83).

Both modified *Fuller's* earth methods (method No. 2 and No. 4) are linear up to a concentration of at least 1770 µmol/l (20 mg/dl) (fig. 4). Higher concentrations could not be measured spectrometrically with sufficient reliability. Therefore, samples with concentrations above 1700 µmol/l should be either diluted or reanalyzed with half of the sample volume. The detection limit determined according to *Kaiser* (16) is about 9 µmol/l.

With control sera (tab. 6) the values obtained with both *Fuller*'s earth methods agreed well with the assigned values. Using Validate N, Lab-trol and Moni-trol I about 5% higher values were found with method No. 4.

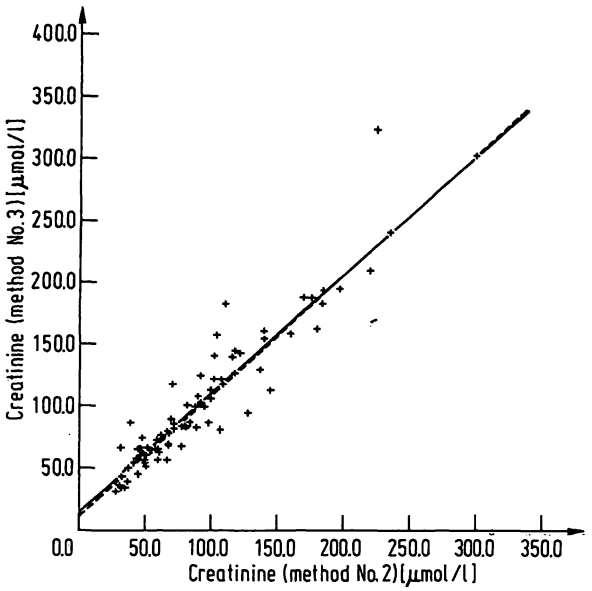

Fig. 3. Comparison of the creatinine concentration in 95 patient sera determined with *Fuller*'s earth procedures with (method No. 2) and without deproteinization (method No. 3). Dotted line = standardized principle component ($y = 0.96x + 12.79$); drawn line = linear regression analysis ($y = 0.95x + 13.83$; $r = 0.99$); $\bar{x} = 120.12$ ($s = 142.34$), $\bar{y} = 128.43$ ($s = 137.03$).

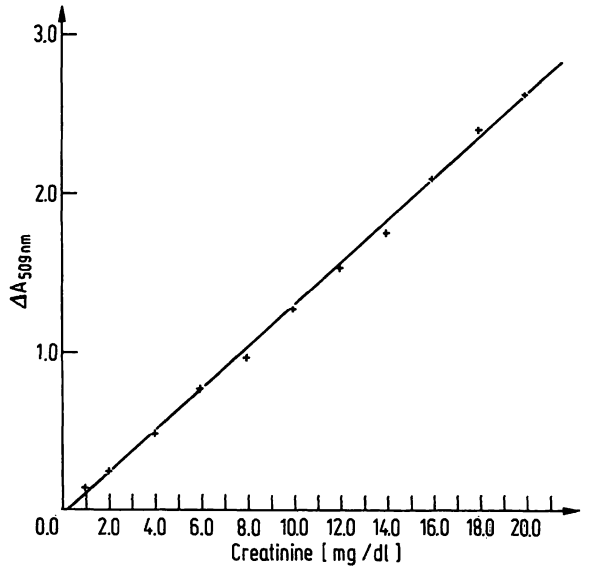

Fig. 4. Relation between creatinine concentration of standard solutions and values measured. Linear regression analysis: $y = 0.998x - 0.029$, $r = 1.00$.

Tab. 5. The influence of lipemia on the creatinine concentration.

Serum No.	Triglyceride-glycerol (mmol/l)	Creatinine (µmol/l) with deproteinization	without deproteinization	SMA-procedure
		method No. 2	method No. 3	method No. 6
1	3.18	48	117	53
2	29.82	412	447	390
3	4.91	333	389	331
4	3.68	104	139	106
5	4.11	70	155	–
6	3.91	120	163	–
7	2.11	93	110	–
8	3.61	96	119	99
9	8.88	53	75	–
10	3.92	44	58	48
11	6.90	68	104	72
12	8.52	78	103	93
mean value (No. 5, 6, 7 and 9 excluded)		147	185	149

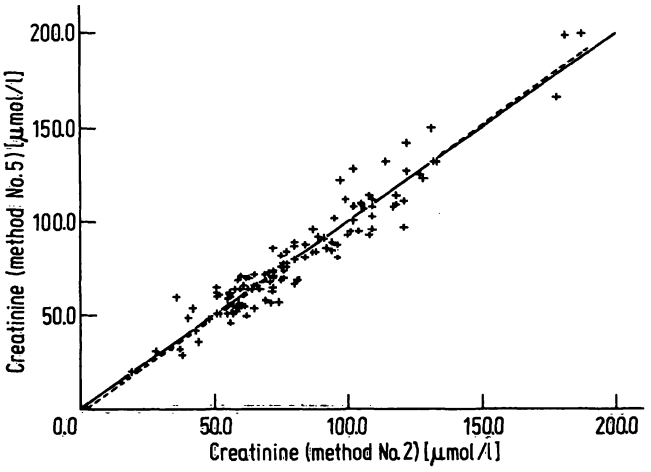

Fig. 5. Comparison of the creatinine concentration in 123 patient sera determined with the fading fraction method (No. 5) and the *Fuller*'s earth procedure with deproteinization as proposed above. Dotted line = standardized principle component ($y = 1.04x - 3.04$). Drawn line = linear regression ($y = 1.00x + 0.55$, $r = 0.96$); $\bar{x} = 80.20$ ($s = 29.65$), $\bar{y} = 80.57$ ($s = 30.91$).

The results of method No. 2 also agreed well with those obtained with the fading fraction- (fig. 5) and with an enzymatic procedure (fig. 6). From the graphical presentation in figure 6 it can be seen that the standardized principle component better describes the scattering of data caused by the variation of both methods than the regression line. All lines were drawn through the ordinate to demonstrate the difference between intercepts.

Recovery experiments showed between 95.5 and 104% recovery of creatinine (tab. 7).

From the results presented so far, method No. 2 was considered as a candidate for a selected method. Therefore, an extensive interference study was undertaken.

Tab. 6. Comparison of the creatinine concentration determined with both *Fuller*'s earth methods modified in various control sera with the assigned values. Each value is a mean of at least 15 determinations on various days.

Control serum (lot No.)	Assigned value[1] (control range) (μmol/l)	Method No. 2 found (μmol/l)	deviation from assigned value, %	Method No. 4 found (μmol/l)	deviation from assigned value, %
Validate N (0610062)	74 (66–83)	77	104	82	111
Lab-trol (38 A-Z)	73 (64–82)	73	100	78	107
Moni-trol I (32 A)	80 (71–89)	84	105	88	110
Moni-trol II (32 A)	309 (274–344)	323	105	325	105
Hyland Europa Kontrolle II (VO 1)	372 (336–408)	399	107	401	108
Behring Richtigkeit (117)	163 (150–176)	165	101	167	102

[1]) according to method No. 1, resp. 2

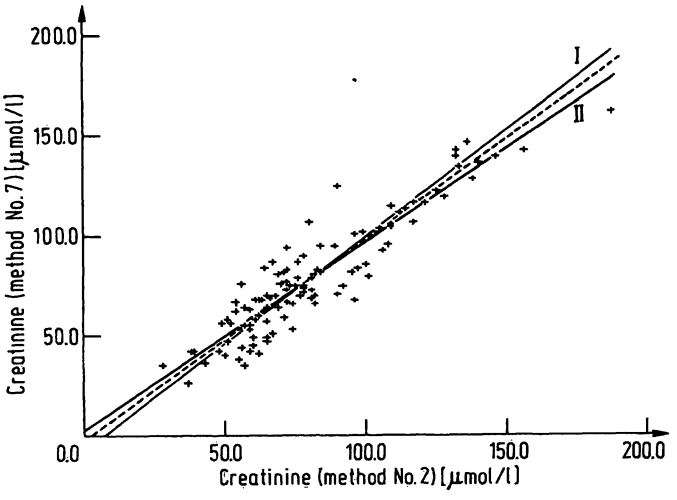

Fig. 6. Comparison of the creatinine concentration in 108 patient sera determined with the *Fuller*'s earth procedure including deproteinization and the enzymatic method. Dotted line ≙ standardized principle component ($y = 1.01x - 2.87$); drawn line = linear regression of 1. order (I, $y = 0.95x + 2.44$; $r = 0.94$; $n = 108$) and of 2. order (II): $\bar{x} = 82.08$ ($s = 31.56$), $\bar{y} = 80.15$ ($s = 31.92$).

Tab. 7. Recovery of creatinine added to various patient sera. Each value is the mean of 2 determinations.

Sample No.	Creatinine calculated 1 + 3 [1]) (μmol/l)	Creatinine found 2 [1]) (μmol/l)	Recovery (%)
A) *Fuller*'s earth procedure with deproteinization (method No. 2):			
1	254	254	100.0
	418	415	99.3
2	274	279	101.8
3	267	255	95.5
4	229	239	104.3
5	519	516	99.4
	350	352	100.6
6	526	532	101.1
B) *Fuller*'s earth procedure without deproteinization (method No. 4):			
1	328	329	100.4
2	319	320	100.3
3	156	156	100.0
4	284	286	100.7
5	232	230	99.1
6	223	226	101.1

[1]) explanation under methods (recovery experiments)

Interferences

Several commonly used drugs were added to human pool-serum (tab. 8) in relatively high concentrations which presumably cannot be expected under therapeutic conditions (17, 18). Nitrofurantoin was the only substance with significant interference. This drug is rapidly excreted into the urine and, therefore, does not accumulate in the blood under therapeutic conditions. Maximal concentrations reported are 5.5 mg/l (19) which did not lead to a measurable absorbance with the method proposed above (tab. 9). Several further substances which have been shown to interfere with the *Jaffé* reaction have also been studied (tab. 9). Pyruvate and 2-oxoglutarate caused a slight positive interference only in method No. 2 and at unphysiologically high concentrations. The novaminsulfone effect is only of theoretical interest, because this substance is rapidly metabolized and cannot be measured in significant blood concentrations (20).

Tab. 8. Recovery of creatinine in human pooled sera containing various drugs. In the absence of any substance added a mean value of 78 μmol/l creatinine was found (n = 10, s = 53, 2s - range = 67 - 87).

Trade name	I. N. N. [1])	Concentration of drug (mg/l)	Creatinine (μmol/l)	
			Fuller's earth procedure	fading fraction method
Glifanan	glafeninum	240	83	77
Aspirin	acidum acetylosalicylicum	600	82	86
Butazolidin	phenylbutazonum	120	78	83
Buscopan	hyoscin-N-butylbrominum	12	80	86
Amuno	indometacinum	30	78	86
Dolviran	acidum acetylosalicylicum, etc.	480	82	82
Prolixan 300	azopropazon-dihydratum	360	76	83
Actol	acidum niflumínicum	150	77	86
Tanderil	oxyphenbutazonum	120	74	86
Metalcaptase	*D*-penicillaminum	480	81	86
Zyloric	allopurinolum	80	77	76
Uricovac	benzbromaronum	20	75	75
Benemid	probenecidum	200	88	77
Lanicor	digoxinum	0.15	86	84
Intensain	carbocromenum	90	83	85
Novadral	norfenefrinum	6	73	83
Miroton	glycosides, etc.	6 ml/l	75	75
Aldactone	spironolactonum	20	77	86
Sembrina	α-methyldopum	320	79	70
Modenol	thiabutazide, etc.	2.6	77	83
Dipar	phenylethylbiguanide	30	83	81
Euglycon	glibenclamidum	3	85	83
Rastinon	tolbutamidum	400	73	72
Solu-Decortin	prednisolonum	200	83	82
Aponal	doxepinum	30	77	85
Librium	chlordiazepoxidum	20	84	84
Methotrexate	acidum methylpteroylglutaminicum	1	80	78
Endoxan	cyclophosphamidum	40	82	81
Megaphen	phenothiazinum	30	80	82
Luminal	acidum phenylbarbituricum	80	76	81
Hostacyclin	tetracyclinum	200	80	81
Paraxin	chloramphenicolum	600	80	79
Binotal	aminobenzylpenicillinum	600	79	
Sulfa-Furadantin	sulfametum	300	82	80
Furadantin	nitrofurantoinum	30	102 [2])	113 [2])
Durenat	sulfanilamidopyrimidinum	200	80	86
Refobacin	gentamycinum	6	81	79
Lasix	furosemidum	20	82	80
Dulcolax	bisacodylum	4	77	79
Angiografin	acidum trijodbenzoicum	4 ml	79	80
Urografin	acidum trijodbenzoicum	4 ml	77	79
Biligrafin	adipinyltrijodanilidum	4 ml	74	73
Resochin	chloroquinum	100	81	81
Polybion	vitamin B complex	0.8 ml	80	78
Nicobion	nicotinamidum	40	79	82
Cebion	acidum ascorbicum	400	78	78
Marcumar	phenprocoumonum	6	82	82
Macrodex	dextranum 6%	100 ml	73	76
Neoplasmagel	gelatine 6%	100 ml	77	83
Anticoagulantia	Na-oxalate	3000	83	85
	Na-fluoride	2000	77	79
	Titriplex III	1000	87	86
	Na-heparinate	750	80	80
	Na-citrate	5000	82	85
Dura-Clofibrat	clofibratum	400	82	83
Antistin	antazolinum	160	75	83

[1]) international non-proprietary names proposed by the WHO
[2]) this value is outside the 2s-range

Tab. 9. The influence of various substances on the determination of the creatinine concentration.

Substance (concentration)	Creatinine (μmol/l)			
	Fuller's earth method with deproteinization (No. 2)		fading fraction method (No. 5)	
	control (no substance added)	interfering substance added	control (no substance added)	interfering substance added
Pyruvate (2 mmol/l)	0	14 [1])	0	92
2-Oxoglutarate (10 mmol/l)	0	47 ± 4 (n = 18) [2])	0	695 ± 118 (n = 16) [2])
Novaminsulfone (1 g/l)	166	165	159	162
(8 g/l)	166	240	159	164
Acetoacetate (2.71 mmol/l)			187	205
(2.30 mmol/l)	161	165		
Nitrofurantoin (5 mg/l)	0	0	0	5
(30 mg/l)	0	28	0	38
(300 mg/l)	0	385	0	354

[1]) μmol/l creatinine (mean values of at least 2 determinations)
[2]) mean value with standard deviation and number of contributing values in parenthesis

An overestimation of creatinine in the presence of acetoacetate by the fading fraction method (tab. 9) has already been observed (21).

Hemoglobin did not interfere (up to 7 g/l), even if the deproteinization step was omitted (tab. 10). In the absence of $MgCl_2$, identical results were obtained with clear patients sera, but not in hemolytic sera (tab. 10). Presumably hemoglobin is bound by $Mg(OH)_2$ which is formed in the final reaction mixture and precipitated by centrifugation.

Urine analyses

Since urine samples are diluted 100 fold with distilled water or physiological saline solutions in most methods,

Tab. 10. The influence of hemolysis on method No. 4 for the determination of the creatinine concentration (μmol/l).

Patient serum No.	$MgCl_2$ in the picrate solution	Creatinine concentration (μmol/l)		Hemoglobin concentration (g/l)
		without hemolysis	with hemolysis	
1	+	66	67	3.7
2	+	84	86	3.8
3	+	86	86	3.8
4	+	83	82	4.2
5	+	64	65	7.2
6	+	71	73	6.8
7	+	76	78	3.6
8	+	88	87	3.4
9	+	86	88	3.6
10	–	67	100	2.9
11	–	75	88	3.9
12	–	75	93	3.6

Tab. 11. Comparison of urine creatinine concentration (mmol/l) determined according to *Taussky* (A) and another procedure with an adsorption step (B). Only those samples were used, which showed a clearance value higher than 200 ml · min^{-1} when determined by with method A during a one month period.

	Creatinine (mmol/l)	
	A	B
1	8.0	6.4
2	10.6	3.5
3	6.5	4.1
4	10.0	8.1
5	11.0	2.8
6	5.2	2.7
7	8.0	5.9
8	14.5	14.4
9	10.6	4.6
10	9.4	3.6
11	9.3	3.0
12	3.7	3.0
13	20.4	21.4
14	9.0	2.6
15	6.2	5.1
16	8.7	2.4
17	21.7	18.2
18	10.9	3.4
19	9.9	1.8
20	11.2	4.0
21	9.5	2.5
22	6.4	2.2
23	8.3	2.1
24	5.1	3.0
25	5.4	3.5
26	14.1	13.2
27	3.8	3.2
28	11.4	2.8
29	5.2	2.9
mean value	9.4	5.4

they are usually subjected directly to the *Jaffé* reaction without prior deproteinization or adsorption to *Fuller*'s earth. However, we regularly noticed samples in which the creatinine concentration was apparently overestimated if the adsorption step was omitted (tab. 11). Similar results were reported by *Sadilek* (22). In 2 cases with three-to fourfold falsely elevated values (in the absence of the absorption step) the reason could be identified. One patient suffered from alcaptonuria, the other one was treated with a mefoxitine monotherapy (cephalosporin, Sharp & Dohme). Cephalosporin antibiotics are also reported to interfere with the creatinine determination in serum procedures without a *Fuller*'s earth separation step (23).

Practicability

The modifications proposed (method No. 2 and No. 4) recommend the use of *Fuller*'s earth suspension dispensed into one-way reaction cups, which can be prepared at any time. In comparison with the method of *Knoll & Stamm*, 6 instead of 11 pipetting steps are required; and compared with the method of *Knoll & Wisser*, there are only 3 instead of 5 pipetting steps.

In our hands No. 2 needs 44 minutes (52 minutes for 10), method No. 4 needs 15 minutes (23 minutes for 10) and the fading fraction method 59 minutes (78 minutes for 10) for 1 unknown sample (1 reagent blank, 1 standard and 1 control sample included).

If motor-driven dispensers are used, the tungstate solution and sulfuric acid can be dispensed simultaneously. Premixing of both reagents is only possible if the mixture is used immediately, but this is not recommended under routine conditions.

Conclusion

The *Knoll & Stamm* modification of *Owen*'s procedure for the determination of the creatinine concentration in serum was further simplified, reducing the pipetting steps and improving precision. The omission of deproteinization leads to a loss of accuracy.

Although this modification correlates very well with the fading fraction and the enzymatic procedure, these methods either require more labour time or are less precise. Therefore, the modified procedure with deproteinization and adsorption of creatinine to *Fuller*'s earth can be considered as a candidate for a selected method according to *Stamm* (24).

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